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1. Document ID: US 20020184662 A1

L3: Entry 1 of 9 File: PGPB Dec 5, 2002

PGPUB-DOCUMENT-NUMBER: 20020184662
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020184662 A1

TITLE: Modified barley alpha-glucosidase

PUBLICATION-DATE: December 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Henson, Cynthia A.	DeForest	WI	US	
Muslin, Elizabeth H.	Madison	WI	US	
Clark, Suzanne E.	Madison	WI	US	

US-CL-CURRENT: 800/284; 435/210, 435/252.3, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

2. Document ID: US 6537792 B1

L3: Entry 2 of 9 File: USPT Mar 25, 2003

US-PAT-NO: 6537792
DOCUMENT-IDENTIFIER: US 6537792 B1

TITLE: Protein engineering of glucoamylase to increase pH optimum, substrate specificity and thermostability

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

3. Document ID: US 6184026 B1

L3: Entry 3 of 9 File: USPT Feb 6, 2001

US-PAT-NO: 6184026
DOCUMENT-IDENTIFIER: US 6184026 B1

TITLE: Morphological mutants of filamentous fungi

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

4. Document ID: US 6066493 A

L3: Entry 4 of 9 File: USPT May 23, 2000

US-PAT-NO: 6066493
DOCUMENT-IDENTIFIER: US 6066493 A
**** See image for Certificate of Correction ****

TITLE: Morphological mutants of filamentous fungi

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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5. Document ID: US 5939305 A

L3: Entry 5 of 9 File: USPT Aug 17, 1999

US-PAT-NO: 5939305
DOCUMENT-IDENTIFIER: US 5939305 A
**** See image for Certificate of Correction ****

TITLE: Gene encoding carboxypeptidase of aspergillus niger

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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6. Document ID: US 5705376 A

L3: Entry 6 of 9 File: USPT Jan 6, 1998

US-PAT-NO: 5705376
DOCUMENT-IDENTIFIER: US 5705376 A

TITLE: Gene encoding carboxypeptidase of Aspergillus niger

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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7. Document ID: US 5693510 A

L3: Entry 7 of 9 File: USPT Dec 2, 1997

US-PAT-NO: 5693510
DOCUMENT-IDENTIFIER: US 5693510 A

TITLE: Gene encoding carboxypeptidase of Aspergillus niger

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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8. Document ID: US 5688663 A

L3: Entry 8 of 9 File: USPT Nov 18, 1997

US-PAT-NO: 5688663
DOCUMENT-IDENTIFIER: US 5688663 A

TITLE: Gene encoding carboxypeptidase of Aspergillus niger

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Image

9. Document ID: US 5594119 A

L3: Entry 9 of 9 File: USPT Jan 14, 1997

US-PAT-NO: 5594119
DOCUMENT-IDENTIFIER: US 5594119 A

TITLE: Gene encoding carboxypeptidase of aspergillus niger

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Image

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#27	Search **crystal structure** Field: **Title/Abstract**	09:27:08	22258
#18	Search **proline AND thermostability** Field: **Text Word**	09:26:36	63
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#15	Search **#13 AND #14** Field: **Pagination,** Limits: **Publication Date from 1989 to 1989**	09:12:21	0
#14	Search **146** Field: **Pagination,** Limits: **Publication Date from 1989 to 1989**	09:12:06	321
#13	Search **Suzuki Y** Field: **Author,** Limits: **Publication Date from 1989 to 1989**	09:11:27	183
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#2	Search **alpha-glucosidase** Field: **Title/Abstract**	08:17:05	2022
#1	Search **alpha-glucosidase**	08:16:38	3282

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1: Biochimie. 1998 Nov;80(11):933-41. Related Articles, Links

Protein thermostability in extremophiles.

Scandurra R, Consalvi V, Chiaraluce R, Politi L, Engel PC.

Dipartimento di Scienze Biochimiche A.Rossi-Fanelli Universita La Sapienza, Rome, Italy.

Thermostability of a protein is a property which cannot be attributed to the presence of a particular amino acid or to a post synthetic modification. Thermostability seems to be a property acquired by a protein through many small structural modifications obtained with the exchange of some amino acids and the modulation of the canonical forces found in all proteins such as electrostatic (hydrogen bonds and ion-pairs) and hydrophobic interactions. Proteins produced by thermo and hyperthermophilic microorganisms, growing between 45 and 110 degrees C are in general more resistant to thermal and chemical denaturation than their mesophilic counterparts. The observed structural resistance may reflect a restriction on the flexibility of these proteins, which, while allowing them to be functionally competent at elevated temperatures, renders them unusually rigid at mesophilic temperatures (10-45 degrees C). The increased rigidity at mesophilic temperatures may find a structural determinant in increased compactness. In thermophilic proteins a number of amino acids are often exchanged. These exchanges with some strategic placement of proline in beta-turns give rise to a stabilization of the protein. Mutagenesis experiments have confirmed this statement. From the comparative analysis of the X-ray structures available for several families of proteins, including at least one thermophilic structure in each case, it appears that thermal stabilization is accompanied by an increase in hydrogen bonds and salt bridges. Thermostability appears also related to a better packing within buried regions. Despite these generalisations, no universal rules can be found in these proteins to achieve thermostability.

Publication Types:
- Review
- Review, Tutorial

PMID: 9893953 [PubMed - indexed for MEDLINE]

1: Biosci Biotechnol Biochem. 1999 Sep;63(9):1535-40. Related Articles, Links

Thermostabilization by proline substitution in an alkaline, liquefying alpha-amylase from Bacillus sp. strain KSM-1378.

Igarashi K, Ozawa T, Ikawakitayama K, Hayashi Y, Araki H, Endo K, Hagihara H, Ozaki K, Kawai S, Ito S.

Tochigi Research Laboratories of Kao Corporation, Japan.
300477@kastanet.kao.co.jp

alpha-Amylase (LAMY) from alkaliphilic Bacillus sp. strain KSM-1378 is a novel semi-alkaline enzyme which has 5-fold higher specific activity than that of a Bacillus licheniformis enzyme. The Arg124 in LAMY was replaced with proline by site-directed mutagenesis to increase thermostability of the enzyme. The wild-type and engineered LAMYs were very similar with respect to specific activity, kinetic values, pH-activity curve, and degree of inhibition by chelating reagents. Thermostability and structure stiffness of LAMYs as measured by fluorescence were increased by the proline substitution. The change of Arg124 to proline is assumed to stabilize the loop region involving amino acid residues from 122 to 134. This is the first report that thermostability of an alpha-amylase is improved by proline substitution.

PMID: 10540739 [PubMed - indexed for MEDLINE]

1: Protein Eng. 1997 Oct;10(10):1199-204. Related Articles, Links

Effect of introducing proline residues on the stability of Aspergillus awamori.

Li Y, Reilly PJ, Ford C.

Department of Zoology and Genetics, Iowa State University, Ames 50011, USA.

In Aspergillus awamori glucoamylase, Ala27, Ala393, Ala435, Ser436 and Ser460 were replaced with proline residues, in order to stabilize the enzyme by forming more rigid peptide backbones. Specific activities were unaffected except for a decrease in Ser460-->Pro glucoamylase. Thermostability was increased in Ser436-->Pro glucoamylase, unchanged in Ala435-->Pro glucoamylase and decreased in Ala27-->Pro, Ala393-->Pro glucoamylases. As measured by circular dichroism, mutant glucoamylases Ala435-->Pro and Ser436-->Pro resisted unfolding caused by guanidine hydrochloride at pH 4.5 and 25 degrees C better than wild-type glucoamylase, whereas mutant glucoamylases Ala27-->Pro, Ala393-->Pro and Ser460-->Pro were more susceptible to unfolding than wild-type glucoamylase, reaching a level of 50% unfolded enzyme at guanidine hydrochloride concentrations 0.50-0.75 M lower than that of the wild-type enzyme. Mutations Ala435-->Pro and Ser436-->Pro are located in a non-regular structure, which is assumed to be stabilized by these mutations. The Ala27-->Pro residue is partially buried, which may result in unfavorable steric contact and/or regional strains; mutation Ala393-->Pro results in loss of a hydrogen bond, since the N of the proline residue does not have an extra hydrogen to act as donor; and mutation Ser480-->Pro eliminates an O-glycosylation site, which could explain how these mutations destabilized glucoamylase.

PMID: 9488144 [PubMed - indexed for MEDLINE]

1: Protein Eng. 2001 Apr;14(4):245-53. Related Articles, Links

Mutant barley (1-->3,1-->4)-beta-glucan endohydrolases with enhanced thermostability.

Stewart RJ, Varghese JN, Garrett TP, Hoj PB, Fincher GB.

Department of Plant Science, University of Adelaide, Waite Campus, Glen Osmond, SA 5064, Biomolecular Research Institute, 343 Royal Parade, Parkville, Vic 3052.

The similar three-dimensional structures of barley (1-->3)-beta-glucan endohydrolases and (1-->3,1-->4)-beta-glucan endohydrolases indicate that the enzymes are closely related in evolutionary terms. However, the (1-->3)-beta-glucanases hydrolyze polysaccharides of the type found in fungal cell walls and are members of the pathogenesis-related PR2 group of proteins, while the (1-->3,1-->4)-beta-glucanases function in plant cell wall metabolism. The (1-->3)-beta-glucanases have evolved to be significantly more stable than the (1-->3,1-->4)-beta-glucanases, probably as a consequence of the hostile environments imposed upon the plant by invading microorganisms. In attempts to define the molecular basis for the differences in stability, eight amino acid substitutions were introduced into a barley (1-->3,1-->4)-beta-glucanase using site-directed mutagenesis of a cDNA that encodes the enzyme. The amino acid substitutions chosen were based on structural comparisons of the barley (1-->3)- and (1-->3,1-->4)-beta-glucanases and of other higher plant (1-->3)-beta-glucanases. Three of the resulting mutant enzymes showed increased thermostability compared with the wild-type (1-->3,1-->4)-beta-glucanase. The largest increase in stability was observed when the histidine at position 300 was changed to a proline (mutant H300P), a mutation that was likely to decrease the entropy of the unfolded state of the enzyme. Furthermore, the three amino acid substitutions which increased the thermostability of barley (1-->3,1-->4)-beta-glucanase isoenzyme EII were all located in the COOH-terminal loop of the enzyme. Thus, this loop represents a particularly unstable region of the enzyme and could be involved in the initiation of unfolding of the (1-->3,1-->4)-beta-glucanase at elevated temperatures.

PMID: 11391016 [PubMed - indexed for MEDLINE]

1: Protein Eng. 1999 Aug;12(8):635-8. Related Articles, Links

Increasing the thermostability of D-xylose isomerase by introduction of a proline into the turn of a random coil.

Zhu GP, Xu C, Teng MK, Tao LM, Zhu XY, Wu CJ, Hang J, Niu LW, Wang YZ.

Department of Molecular Biology and Cell Biology and The Key Lab of Structural Biology, USTC, CAS, School of Life Science, University of Science and Technology of China, Hefei, Anhui 230026, China.

Thermostability can be increased by introducing prolines at suitable sites in target proteins. Two single (G138P, G247D) mutants and one double (G138P/G247D) mutant of xylose isomerase from Streptomyces diastaticus No.7, strain M1033 have been constructed by site-directed mutagenesis. With respect to the wild-type enzyme, G138P showed about a 100% increase in thermostability, and G247D showed an increased catalytic activity. Significantly, the double mutant, G138P/G247D displayed even higher activity than G247D and better heat stability than G138P. Its half life was about 2.5-fold greater than the wild-type enzyme, using xylose as a substrate. Molecular modelling suggested that the introduction of a proline residue in the turn of a random coil may cause the surrounding conformation to be tightened by reducing the backbone flexibility. The change in thermostability can, therefore, be explained based on changes in the molecular rigidity. Furthermore, the improvements in the properties of the double mutant indicated that the advantages of two single mutants can be combined effectively.

PMID: 10469823 [PubMed - indexed for MEDLINE]

1: Eur J Biochem. 1994 Mar 1;220(2):293-300. Related Articles, Links

Structure and expression of a gene coding for thermostable alpha-glucosidase with a broad substrate specificity from Bacillus sp. SAM1606.

Nakao M, Nakayama T, Kakudo A, Inohara M, Harada M, Omura F, Shibano Y.

Institute for Biomedical Research, Suntory Ltd., Osaka, Japan.

We cloned an alpha-glucosidase gene from thermophilic Bacillus sp. SAM1606 to overexpress it in Escherichia coli transformants. Deletion of the 5'-noncoding region as well as expression of the alpha-glucosidase gene under the control of the icp promotor of the insecticidal crystal protein gene from Bacillus thuringiensis subsp. sotto enhanced the enzyme productivity to 23.5 U/ml, which was 12,000-fold higher than that obtained by the strain SAM1606. The open reading frame corresponding to the alpha-glucosidase encoded 587 amino acid residues including a residue coded by the initiation codon TTG, and the molecular mass of the alpha-glucosidase from N-terminal serine was calculated to be 68,886 Da. Sequence analysis revealed that the SAM1606 alpha-glucosidase belonged to the alpha-amylase family. The SAM1606 alpha-glucosidase showed extremely high sequence identity (62-65%) to the Bacillus cereus and Bacillus thermoglucosidasius oligo-1,6-glucosidases, which were 72% identical to each other. Sequence identity in the suggested active site regions were essentially the same (80-82%) among these three enzymes. However, the substrate specificity of the SAM1606 alpha-glucosidase was significantly different from those of the oligo-1,6-glucosidases. The thermostability of these three alpha-glucosidases could be correlated with the increase in the number of proline residues, whose occurrence was predicted at beta turns and coils in the enzymes.

PMID: 8125087 [PubMed - indexed for MEDLINE]

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The effect of proline insertions on the thermostability of a barley alpha-glucosidase.
Protein Eng. 2002 Jan;15(1):29-33.
PMID: 11842235 [PubMed - indexed for MEDLINE]

2: Boisson M, Gomord V, Audran C, Berger N, Dubreucq B, Granier F, Lerouge P, Faye L, Caboche M, Lepiniec L. Related Articles, Links

Arabidopsis glucosidase I mutants reveal a critical role of N-glycan trimming in seed development.
EMBO J. 2001 Mar 1;20(5):1010-9.
PMID: 11230125 [PubMed - indexed for MEDLINE]

3: Taylor MA, George LA, Ross HA, Davies HV. Related Articles, Links

cDNA cloning and characterisation of an alpha-glucosidase gene from potato (Solanum tuberosum L.).
Plant J. 1998 Feb;13(3):419-25.
PMID: 9680991 [PubMed - indexed for MEDLINE]

1: Protein Eng. 2002 Jan;15(1):29-33. Related Articles, Links

The effect of proline insertions on the thermostability of a barley alpha-glucosidase.

Muslin EH, Clark SE, Henson CA.

Department of Agronomy, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706, USA.

The thermal stability of alpha-glucosidase is important because the conversion of starch to fermentable sugars during industrial production of ethanol (e.g. brewing, fuel ethanol production) typically takes place at temperatures of 65-73 degrees C. In this study we investigate the thermostability of alpha-glucosidases from four plant species, compare their deduced amino acid sequences, and test the effect of substituting a proline for the residue present in the wild-type enzyme on the thermostability of alpha-glucosidase. The alpha-glucosidase from barley (Hordeum vulgare) was significantly less thermostable than the other three alpha-glucosidases. A comparison of the published deduced amino acid sequences of these four alpha-glucosidases revealed conserved proline residues in the three most thermostable alpha-glucosidases that were not found in the barley enzyme. Site-directed mutagenesis was done on recombinant barley alpha-glucosidase to create proteins with prolines at these conserved positions. The thermostability (T(50)) of one of these mutant enzymes, T340P, was 10 degrees C higher than the non-mutated enzyme.

PMID: 11842235 [PubMed - indexed for MEDLINE]

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1: Gomes I, Gomes J, Steiner W. Related Articles, Links

Highly thermostable amylase and pullulanase of the extreme thermophilic eubacterium Rhodothermus marinus: production and partial characterization.
Bioresour Technol. 2003 Nov;90(2):207-14.
PMID: 12895565 [PubMed - in process]

2: McCleary BV, McNally M, Monaghan D, Mugford DC. Related Articles, Links

Measurement of alpha-amylase activity in white wheat flour, milled malt, and microbial enzyme preparations, using the Ceralpha assay: collaborative study.
J AOAC Int. 2002 Sep-Oct;85(5):1096-102.
PMID: 12374409 [PubMed - indexed for MEDLINE]

3: Bertoldo C, Antranikian G. Related Articles, Links

Starch-hydrolyzing enzymes from thermophilic archaea and bacteria.
Curr Opin Chem Biol. 2002 Apr;6(2):151-60. Review.
PMID: 12038998 [PubMed - indexed for MEDLINE]

4: Muslin EH, Clark SE, Henson CA. Related Articles, Links

The effect of proline insertions on the thermostability of a barley alpha-glucosidase.
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PMID: 11842235 [PubMed - indexed for MEDLINE]

5: Watanabe K, Fujiwara H, Inui K, Suzuki Y. Related Articles, Links

Oligo-1,6-glucosidase from a thermophile, Bacillus thermoglucosidasius KP1006, was efficiently produced by combinatorial expression of GroEL in Escherichia coli.
Biotechnol Appl Biochem. 2002 Feb;35(Pt 1):35-43.
PMID: 11834128 [PubMed - indexed for MEDLINE]

6: Schiraldi C, Martino A, Acone M, Di Lernia I, Di Lazzaro A, Marulli F, Generoso M, Carteni M, De Rosa M. Related Articles, Links

Effective production of a thermostable alpha-glucosidase from Sulfolobus solfataricus in Escherichia coli exploiting a microfiltration bioreactor.
Biotechnol Bioeng. 2000 Dec 20;70(6):670-6.
PMID: 11064336 [PubMed - indexed for MEDLINE]

7: Raasch C, Streit W, Schanzer J, Bibel M, Gosslar U, Liebl W. Related Articles, Links

Thermotoga maritima AglA, an extremely thermostable NAD+-, Mn2+-, and thiol-dependent alpha-glucosidase.
Extremophiles. 2000 Aug;4(4):189-200.
PMID: 10972187 [PubMed - indexed for MEDLINE]

8: Ganghofner D, Kellermann J, Staudenbauer WL, Bronnenmeier K. Related Articles, Links

Purification and properties of an amylopullulanase, a glucoamylase, and an alpha-glucosidase in the amylolytic enzyme system of Thermoanaerobacterium thermosaccharolyticum.
Biosci Biotechnol Biochem. 1998 Feb;62(2):302-8.
PMID: 9532787 [PubMed - indexed for MEDLINE]

9: Bibel M, Brettl C, Gosslar U, Kriegshauser G, Liebl W. Related Articles, Links

Isolation and analysis of genes for amylolytic enzymes of the hyperthermophilic bacterium Thermotoga maritima.
FEMS Microbiol Lett. 1998 Jan 1;158(1):9-15.
PMID: 9453151 [PubMed - indexed for MEDLINE]

10: Wimmer B, Lottspeich F, Ritter J, Bronnenmeier K. Related Articles, Links

A novel type of thermostable alpha-D-glucosidase from Thermoanaerobacter thermohydrosulfuricus exhibiting maltodextrinohydrolase activity.
Biochem J. 1997 Dec 1;328 (Pt 2):581-6.
PMID: 9371718 [PubMed - indexed for MEDLINE]

11: Suzuki Y, Nobiki M, Matsuda M, Sawai T. Related Articles, Links

Bacillus thermoamyloliquefaciens KP1071 alpha-glucosidase II is a thermostable M(r) 540,000 homohexameric alpha-glucosidase with both exo-alpha-1,4-glucosidase and oligo-1,6-glucosidase activities.
Eur J Biochem. 1997 Apr 1;245(1):129-36.
PMID: 9128733 [PubMed - indexed for MEDLINE]

12: Berger JL, Lee BH, Lacroix C. Related Articles, Links

Identification of new enzyme activities of several strains of Thermus species.
Appl Microbiol Biotechnol. 1995 Dec;44(1-2):81-7.
PMID: 8579838 [PubMed - indexed for MEDLINE]

13: Nakao M, Nakayama T, Harada M, Kakudo A, Ikemoto H, Kobayashi S, Shibano Y. Related Articles, Links

Purification and characterization of a Bacillus sp. SAM1606 thermostable alpha-glucosidase with transglucosylation activity.
Appl Microbiol Biotechnol. 1994 May;41(3):337-43.
PMID: 7764968 [PubMed - indexed for MEDLINE]

14: Nakao M, Nakayama T, Kakudo A, Inohara M, Harada M, Omura F, Shibano Y. Related Articles, Links

Structure and expression of a gene coding for thermostable alpha-glucosidase with a broad substrate specificity from Bacillus sp. SAM1606.
Eur J Biochem. 1994 Mar 1;220(2):293-300.
PMID: 8125087 [PubMed - indexed for MEDLINE]

15: Lavrenova TP, Presnova VN. Related Articles, Links

Rat liver neutral alpha-glucosidase: isolation and characterization.
Biochem Mol Biol Int. 1994 Mar;32(4):671-9.
PMID: 8038718 [PubMed - indexed for MEDLINE]

16: Yang S, Zhang S. Related Articles, Links

[Purification and characterization of alpha-glucosidase from an extreme thermophile, Thermus thermophilus HB 8]
Wei Sheng Wu Xue Bao. 1992 Feb;32(1):23-9. Chinese.
PMID: 1413735 [PubMed - indexed for MEDLINE]

17: Banno Y, Sasaki N, Yoshino T, Mochizuki J, Hirata H, Nozawa Y. Related Articles, Links

A thermostable acid alpha-glucosidase from Tetrahymena thermophila: purification and characterization.
J Protozool. 1989 Nov-Dec;36(6):562-7.
PMID: 2689637 [PubMed - indexed for MEDLINE]

18: Alhadeff JA, Thom D, Holzinger RT. Related Articles, Links

Activity levels and properties of acid alpha-glucosidase from liver and neutral alpha-glucosidase from sera of cystic fibrosis patients and controls.
Clin Chim Acta. 1981 Dec 9;117(2):227-37.
PMID: 7030525 [PubMed - indexed for MEDLINE]

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